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Administrative Report H-00-01

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HEALTH ASSESSMENT AND DISEASE STATUS STUDIES OF THE HAWAIIAN MONK SEAL (*MONACHUS SCHAUINSLANDI*)

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INTRODUCTION

The endangered Hawaiian monk seal (*Monachus schauinslandi*) (HMS) population has declined 60% since the first range-wide surveys in the late 1950s and will likely continue to decline as a result of high juvenile mortality and low reproductive recruitment occurring at French Frigate Shoals (FFS), where the largest subpopulation is located (Gilmartin, 1983). If this trend continues, it is conceivable that this endangered species could be on the verge of extinction. Understanding the potential role of disease and toxins in this recent decline is a high priority. Several natural sources of mortality have been identified, including ciguatera poisoning, starvation, shark predation, male aggression (i.e., mobbing), parasitism, and disease. However, the relative significance of these factors and their effect on population trends are poorly understood (Gilmartin, 1993a). Efforts to enhance the recovery of monk seals will require a better understanding of the health status of the wild population.

Since 1981, captive care and release programs have been an integral part of management efforts to enhance the recovery of the Hawaiian monk seal (HMS). Three strategies have been used including 1) on-site protection and release, 2) direct translocation from one site to another, and 3) transport to Oahu for rehabilitation followed by release into the wild population. Initial captive care and release efforts were intended to enhance the depleted subpopulation at Kure Atoll. These efforts were notably successful, and this subpopulation appears to be in the process of recovery. In the late 1980s and early 1990s, juvenile survival at FFS declined severely, and the objective of captive care and release efforts was expanded to include salvaging the reproductive potential being lost at FFS (Ragen and Lavigne, 1998).

In 1995, 12 female pups were captured and brought to Oahu for rehabilitation and eventual release into the wild population. These seals were not released as anticipated due to the development of a persistent eye condition of unknown etiology during the holding period. This unexpected event resulted in a hiatus of the monk seal captive care and release program, and provided the impetus for a review of program activities by a panel of independent wildlife experts (*Captive Care Review Panel Report*, 1997). The panel concluded that translocation is potentially a valuable management tool. Any translocation activity must achieve the goal of supplementing a depleted subpopulation without compromising the potential for growth of the source subpopulation, thereby improving recovery of the total population. The management strategy most likely to achieve the goal of recovery is the direct translocation of larger sized pups from subpopulations with low pup survival (e.g., FFS) to subpopulations with high pup survival (e.g., Midway Atoll). It was recommended that the strategy for the immediate future is translocation with or without conditioning on the beach at the capture or release sites. It is important to determine whether these efforts pose significant risks of introducing pathogens into naive subpopulations and to minimize the threat of any potentially catastrophic disease outbreaks resulting from these recovery efforts.

A strategy for minimizing the risk of introducing diseases to a population during translocation has been described in the Hawaiian Monk Seal Epidemiology Plan (Aguirre et al., 1999). Essentially, this plan consists of screening potential candidates for translocation using the best available diagnostics to test seals for evidence of infection or disease. A second component is the definition of current prevalence rates of infectious diseases or exposure to infectious agents for subpopulations which may serve as donor or recipients during translocation using serologic screening. A third component involves taking a proactive approach to disease management (i. e., treatment or immunization as technology becomes available). Currently, the impacts of disease or subclinical health problems on the declining survival of the monk seal population are unknown. Therefore, this health assessment of the HMS was conducted in order to obtain baseline information on selected health and disease parameters from three Hawaiian monk seal subpopulations.

The objectives of the health assessment were to:

(1) Establish normal baseline values for morphometric, hematologic, and biochemical parameters within age and gender classes of the species at FFS, Midway Atoll (MID) and Pearl and Hermes Reef (PHR) and compare the values for each parameter among adult seals from FFS and PHR.

(2) Determine evidence of exposure to specific marine mammal infectious agents using currently available serologic techniques.

(3) Attempt isolation of *Salmonella* spp. and other pathogenic enteric bacteria and pathogenic viruses from specimens collected at three sites.

(4) Identify parasite exposure and species of parasites present in fecal material collected at three sites.

(5) Determine the concentrations of chlorinated hydrocarbon pesticides and polychlorinated biphenyls (PCBs) in tissues of seals at three sites and determine whether any differences in body burdens of contaminants exist across subpopulations of HMS.

(6) Provide recommendations regarding translocation strategies between surveyed subpopulations at FFS, PHR, and MID.

MATERIALS AND METHODS

Study Sites

This baseline epidemiological study of the Hawaiian monk seal was designed to assess the health and disease status of three breeding subpopulations (donor, recipient, and control)

currently under consideration for enhancement activities by National Marine Fisheries Service (NMFS), Honolulu Laboratory. FFS (23°45'N; 166°10'W) has been considered the donor subpopulation because it is the largest subpopulation and has a low survival of juveniles. MID (28°15'N, 177°23'W) represents the potential recipient subpopulation due to its severely depleted status (11 pups born in 1997), and PHR (27°50'N, 175°50'W) has been selected as a control subpopulation since it is currently growing at a rate of about 5% annually (Aguirre et al., 1999).

Sample Collection

Physical examinations were performed by the attending veterinarian and were limited to visual identification of abnormalities; determination of body condition, sex, size, and class and evidence of trauma. Prior to capture, seals were observed for several minutes to measure respiratory and cardiac rates from a distance. Seals were then captured while hauled out on the beach. Mature seals (subadults and adults) were captured with a hoop net, immature seals (juveniles) were captured with a stretcher, and weaned pups were captured by hand. Diazepam (Steris Laboratories Inc., Phoenix, Arizona) was given intravenously and following induction, cardiac rate, respiratory rate, and rectal temperature (digital thermometer, Fisher Scientific, Pittsburgh, Pennsylvania) were recorded at least once during the handling procedure. Seals were flipper-tagged, PIT-tagged, and scars/marks were recorded for individual identification. Dorsal standard length (L) and axillary girth (G) were recorded with a tape measure. Mass was calculated using a simple mass-dependent formula (G^2 X L X 0.00005) applied for monk seals (Craig and Ragen, 1999). Age, sex, and size classes were determined by reviewing previous identification cards on file or by visual examination at the time of capture. Following all biomedical and morphometric procedures, seals were released and monitored post-capture for 10 to 60 minutes or until normal behavior was observed. An attempt to observe seals days or weeks later was made to identify obvious clinical signs of disease or other abnormalities.

Blood (35 to 60 ml) was collected from the bilaterally divided extradural veins by inserting an 18 ga, 3.5 in spinal needle between the dorsal spinous processes of the 3rd, 4th, or 5th lumbar vertebrae (Geraci and Lounsbury, 1993). Blood specimens were immediately transferred into EDTA (3 mL), heparinized (7 mL), and SST (25 mL) Vacutainer® tubes (Becton-Dickinson and Co., Rutherford, New Jersey, USA). EDTA tubes were placed in blue ice, and SSTs were kept in the shade at ambient temperature for 60 minutes to allow normal coagulation process and then transferred to a cooler with blue ice. Plasma and serum specimens were separated by centrifugation at 2,000 rpm for 10 minutes, pipetted off in one-mL aliquots in cryogenic vials (Nalgene), and placed in liquid nitrogen. Specimens collected for biochemistry and serology were ultrafrozen on liquid nitrogen in the field and transferred to a –86°C ultrafreezer prior to analysis upon arrival in Honolulu.

Buffy coats from heparinized and EDTA tubes and rectal swabs were collected for virus isolation. Buffy coats were placed in 1-ml cryogenic vials and immediately frozen in liquid nitrogen. Rectal swabs were placed into 5-ml cryovials containing 2 ml of cell culture medium (Eagle MEM; Whittaker M. A. Bioproducts, Walkersville, Maryland, USA) with 10% fetal calf

serum and 100 mg/ml gentamacine. These samples were also immediately placed in liquid nitrogen and then transferred to the ultrafreezer upon arrival to Honolulu until laboratory testing. Rectal swabs for *Salmonella* analysis from all seals were collected and immediately placed in Modified Cary Blair Transport Medium (Para-Fix, Medical Chemical Corp., Santa Monica, California, USA). All swabs were preserved on blue ice and then transferred to a refrigerator (4° C) until testing. Fecal specimens from seals at all sites were collected with a fecal loop. Samples were placed in a vial containing 5-mL of polyvinyl alcohol fixative solution (PVA) for parasitoscopic screening.

Blubber biopsies and blood from adult males and blood from seals of other size categories were collected for contaminant analysis. Blubber biopsies were collected following a modification of the technique described by Iverson et al. (1997). Briefly, the area was locally blocked with 4-6 ml of Xylocaine 1% (Astra USA Inc., Westborough, Massachusetts), then surgically scrubbed with 70% ethanol and povidone iodine solution. A 2-cm incision including skin and subcutaneous tissue was performed to expose the blubber layer. Blubber core samples were collected from the pelvic region using 6-mm biopsy punches. Biopsy cores (3-4 cm) were taken through the full depth of the blubber layer. Blubber cores were removed with forceps and scissors, placed into 4-ml amber vials, and then frozen at -20°C until being shipped to the laboratory. The wound was then cleansed and not sutured to allow healing by second intention. A sample of whole blood (5-6 ml) from all seals was placed in methylene-chloride-rinsed glass containers and then frozen at -20°C or colder as soon as possible following collection.

Hematology

In the laboratory, EDTA tubes were warmed to room temperature and gently mixed. Pack cell volume (PCV) was determined with a microhematocrit centrifuge and reader (StatSpin, Norwood, Massachusetts). Absolute white blood cell counts were performed using the Microcollection System Test 5804/5853 (UNOPETTE, Becton-Dickinson and Co., Rutherford, New Jersey, USA) within 1-6 hours of blood collection. Blood smears were prepared in duplicate with fresh whole blood and air dried. These were stained with Wright's Giemsa within 8-10 weeks to determine differential white blood cell counts and hemoparasites. Differential white blood cell counts and percentage of each leukocyte type were performed by D. Borjesson, Department of Clinical Pathology, University of California-Davis. Nucleated cell estimates were performed using a high power binocular microscope, and a lower and upper range number was given (1,500-cell range). These values correlated reasonably well with the absolute leucocyte counts performed with the Unopette system when 10% was added as a correction factor. Whole blood was used to measure total solids with a refractometer, glucose with a glucometer, and blood urea nitrogen (BUN) with BUN reagent strips (Quickchek, Centaur, Overland Park, Kansas).

Serum Biochemistry

Biochemistry analysis of serum was performed at IDDEX Veterinary Services (IDDEX, Sacramento, California, USA). Serum biochemistries were determined using an automated random access analyzer. Hemolyzed samples were not included in this study. The following serum determinants were measured: total protein, albumin, globulin, total bilirubin, direct and indirect bilirubin, alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), alkaline phosphatase (AP), creatinine kinase (CK), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), BUN, creatinine, calcium, phosphorus, bicarbonate, cholesterol, triglycerides, glucose, sodium, potassium, chloride, A/G ratio, B/C ratio, Na/K ratio, and anion gap.

Atrial Natriuretic Factor

The measurement of immunoreactive-atrial natriuretic factor (ANF) in the monk seal samples was accomplished using a radioimmunoassay (RIA) specific for human-ANF-(99-126). Radioimmunoassays to measure peptides from the N-terminus of the ANF 126 a.a. prohormone were developed to amino acids 1-30 (proANF 1-30), 31-67 (proANF 31-67), and 79-98 (proANF 79-98) of this prohormone as previously described (Vesely et al.,1989, 1994; Winters et al., 1989).

Serologic Testing

One- to two-ml aliquots of ultrafrozen serum were sent to NMFS certified diagnostic laboratories to determine previous exposure or levels of antibodies to selected infectious agents known to occur in other marine mammal populations (Table 1).

In the Laboratory for Calicivirus Studies (LCS, Oregon State University, Corvallis, Oregon, USA), sera were heat inactivated at 56°C for 30 minutes in preparation for group antibody and serum neutralization (SN) testing against caliciviruses including San Miguel sea lion virus (SMSV) types 1 to 17, walrus calicivirus 7420, feline calicivirus F-9, W-6 calicivirus, VESV A_{48}, primate calicivirus strain, mink calicivirus strain MV 20-3, reptile calicivirus strain 002, cetacean calicivirus strain 041, bovine calicivirus BCV Bos-1, mystery pig disease calicivirus strain P42BN, canine calicivirus strain 731, Orytologus calicivirus, Hawaiian (temporary designation), McAll human isolate, cheetah calicivirus, fur seal herpes virus, walrus adenovirus-1, human herpesvirus-2, walrus retrovirus-like virus, walrus enterovirus-like virus, and pinniped rotavirus-like virus. A microtiter seroneutralization (SN) test procedure using Vero cells was performed, first to screen sera at a dilution of 1:100 and then to titrate positive samples using twofold dilutions (Smith et al., 1976). Serum virus mixtures were incubated for 60 minutes at room temperature before addition of the cells as previously described. The antibody titer was defined as the highest dilution of serum completely neutralizing 100 tissue culture infectious doses-50% ($TCID_{50}$) of virus in all four replicate test wells (100% end point).

Specific rabbit antiserum and more recently, Hawaiian monk seal antiserum were used as a positive control, and type specificities were monitored in parallel neutralization tests during end-point titrations.

Serum samples were tested using SN at the Foreign Animal Disease Diagnostic Laboratory (FADDL, Plumb Island, New York, USA) for the following viruses: vesicular exanthema of swine viruses (VESV) A48, B51, C52, D53, E54, F55, G55, H54, I55, J56, K54, 1934B; SMSV types 1 to 2 and 4 to 13. All viruses were from the FAADL repository except for SMS viruses that were provided by A. Smith (LCS). In addition, canine distemper virus (CDV), phocine distemper virus (PDV), and seal influenza virus were also tested. At FADDL, samples were thawed, diluted 1:10 in sterile Eagles minimum essential salt media (EMEM) containing 25 µg/ml gentamicin (Gensia Pharmaceuticals Inc., Irvine, California, USA) and 2.7 µg/ml amphotericin B (Gensia Pharmaceuticals, Inc.), and inactivated at 56°C for 30 minutes. A screening microtiter virus neutralization test for antibody to caliciviruses was performed in 96 well tissue culture plates (Costar, Cambridge, Massachusetts, USA) using 25 µl serum diluted 1:10 in each of six wells. Then, 25 µl virus suspension containing 100 $TCID_{50}$ was added to three of the wells; the remaining three wells served as controls to detect the serum toxicity. The final dilution of serum in the well was 1:20 for the screening test. After 1 hour incubation at 37° C at 5% CO_2 and 95% humidity, 100 ml of Vero or IBRS-2 cells suspension at 200,000 cells/ml EMEM containing 10% fetal bovine serum were added. Plates were incubated as above for 3 days and examined microscopically for the presence of cytopathic effects. Positive samples to the screening test were titrated and diluted twofold, and the test was repeated for the selected virus. Serum titers were calculated using the Spearman-Karber (S-K) method, giving 50% endpoints, and also by the Monto and Bryan method giving 100% protection endpoints. The serologic test protocol for both morbilliviruses was identical except that 50 µl of diluted serum was mixed with 50 µl virus at 100 $TCID_{50}$/50 µl, and the test was incubated 7 days before reading (O'Hara et al., 1998). In addition, antibodies for seal influenza were tested using the agar gel method.

Ultrafrozen serum specimens were tested at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL, Oklahoma State University, Stillwater, Oklahoma, USA) for the presence of antibodies to four morbilliviruses including canine distemper virus (CDV), phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV) using SN tests following protocols developed by FADDL. The DMV, PDV, and PMV stocks were obtained from FADDL. In addition, specimens were tested for the presence of Phocine herpesvirus 1 with a SN test developed at OADDL.

At OADDL, serum samples were tested for the presence of *Brucella canis* and *B. abortus* antibodies using the standard card agglutination test (SCA). Positive samples then were referred to the State/Federal Brucellosis Laboratory (Oklahoma City, Oklahoma). These were retested using the SCA, the particle concentration fluorescence immunoassay (PCFIA), the brucella buffered antigen standard plate agglutination test (BAPA), the complement fixation test (CF), and the Rivanol test, as previously described (Stemshorn et al., 1985). All procedures followed standard protocols developed by the National Veterinary Services Laboratory (NVSL, Ames,

Iowa). A serum titer was considered positive when all tests--SCA, PCFIA, BAPA, CF, and Rivanol (> I:50) were positive. A seal with serum titers positive to one or more tests but not all tests was considered suspect. Ninety-three serum specimens were also tested at FADDL by the SCA; however, positive specimens were not confirmed with the other methods.

Serum samples were tested for antibody presence of *Chlamydia psittaci* by complement fixation (CF 1:10) following NVSL protocols and procedures. Specimens demonstrating a CF titer $\geq$ 1:20 were considered positive. *Leptospira* screening was performed at the LCS using the microscopic agglutination test (MAT) for group antibodies using *L. pomona* antigen at a test dilution of 1:10 (Cole et al., 1973). Each sample was diluted with 0.85% NaCl and titrated to an end point (dilution showing 50% agglutination) using a series of doubling solutions at 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200. *Leptospira pomona* antigen was used due to the fact that Difco Laboratories discontinued the pooled antigens sales which had been in routine use for over 30 years. This antigen has demonstrated reactivity against serovars *pomona* (Pomona), *icterohaemorrhagica*, *grippothyphosa* (Moskva V), *autumnalis*, *ballum*, and *serjroe*.

Antibodies for the presence of *Toxoplasma gondii* were tested by J. P. Dubey (Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, Agricultural Research Service, USDA, Beltsville, Maryland) using the MAT, latex agglutination test (LAT). Sera were originally screened at 1:25, 1:50, and 1:500 dilutions using mercaptoethanol incorporated in formalin-fixed whole tachyzoites as previously described (Dubey and Desmonts, 1987). Sera with antibodies were titrated in twofold dilutions. The LAT was performed with a commercial test kit following the manufacturer instructions (TOXOTEST-MT, Eiken Chemical Co., San Diego, California, USA). Titers $\geq$ 1:25 were considered positive or considered indicative of previous exposure.

Serum specimens were tested at IDDEX for the presence of antibodies to *Dirofilaria immitis* using an ELISA *D. immitis* antigen test (DiroCHECK, Synbiotics Corporation, San Diego, California, USA).

Virus Isolation, Identification, and Characterization

Rectal swabs were examined for the presence of virus at LCS. Specimens were thawed, vortexed, and clarified by low-speed (3,000 X g for 20 minutes) centrifugation and 0.2 ml of supernatant was adsorbed for 60 minutes to monolayers of PK-15 and Vero cells (American Type Culture Collection, Rockville, Maryland, USA) in roller tubes and processed as previously described (Smith et al., 1980). The inocula were poured off, the monolayers rinsed with cell culture medium (Eagle MEM), and the cells re-fed with MEM and 2% fetal bovine serum and incubated at 37°C on a roller aparatus. Each culture was examined daily for cytopathic effects. All samples were blind-passaged three times, at 7-day intervals, before being considered negative. Virus-like particles causing cytopathic effects were purified as described by Smith et al., 1983. Morphologic features of purified isolates were examined by negative-contrast electron microscopy (Skilling et al., 1985). Second and third passage materials in Vero and PK_{15} cells

from rectal swabs that were initially positive by immunoblot were run and tested again against the same calicivirus-specific monoclonal antibody (4AD8D8). Buffy coats were layered over monolayers to examine them for cell-associated virus and then freeze-lyzed and processed in the same manner as the swab specimens.

Bacterial Isolations

Rectal swabs were tested for isolation and identification of *Salmonella* and other enterobacteria to the Microbiology Department (Veterinary Medical Teaching Hospital, University of California, Davis, California, USA). In the laboratory, swabs were streaked onto MacConkey plates (PML Microbiologicals, Tualatin, Oregon, USA). Plates were incubated aerobically for 24-48 hours at 37°C. Isolated organisms were identified with the API Systems (API Biomerieux Vitek, Hazelwood, Missouri, USA). *Salmonella* isolates were sent to NVDL for serotyping.

Parasitology

PVA-fixed fecal specimens were brought to the NMFS Kewalo Research Facility, Diamond Head Laboratory and analyzed for the presence of helminth ova and adults by M. Kliks (CST Foundation, Honolulu, Hawaii, USA). Fecal samples were analyzed by preparing a standard 2-mg fecal smear from the undiluted sample and microscopically examined for parasite ova and cysts or spores. The remainder of the specimen was commutated in formol saline, passed through three graded sieves, the smallest of which had an opening of 750 m. All retained materials were examined grossly and with the aid of a dissecting microscope for any parasite stages. All candidate objects were stored in glass screw top vials in 70% alcohol plus 5% glycerin for further study; all other solid residues were returned to their original containers in their original fixative solutions. For further study of adult nematode specimens, these were processed into pure glycerin, mounted on labeled microscope slides under a coverslip, and studied under low power using a compound microscope. Cestodes and acanthocephalan worms were stained in aqueous carmine solution, destained in acid alcohol, dehydrated through absolute alcohol, cleared in xylene, and mounted in Canadian balsam on slides under coverglasses. All unmounted and mounted specimens have been processed and stored for further taxonomic studies.

Toxicology

Blubber and blood specimens were analyzed for selected chlorinated hydrocarbons (CHs) including dioxin-like chlorobiphenyls (CBs), 1,1,1-trichloro-2,2-bis(*p* chlorophenyl)ethane (DDT) and its metabolites (e.g., DDEs, DDDs), by rapid high-performance liquid chromatography photodiode array (HPLC/PDA) method by NMFS Environmental Conservation Division (ECD), Seattle, Washington (Khran et al., 1994).

Statistical Analysis

Demographic information, morphological variables, and hematological and biochemistry values were merged into a single SAS file for statistical analysis. A number of seals were captured multiple times leading to multiple records for each seal. For the purpose of this study, data were reduced to one record per seal. The data obtained during the first capture were used to avoid bias in the event that stress experienced by the animals during capture may affect some of the measurements. However, in seven cases all observations from the first capture were missing for one or more of the three data sets. Five of these animals were recaptured within 1-2 weeks, and data from the second capture were used to replace missing data from the first capture.

Monk seals were stratified by gender and grouped into three age classes: "weanlings," "juveniles" (which included young and older juveniles as well as young subadults), and "adults" (which included older subadults and adults). Descriptive statistics (mean, standard deviation, median, minimum, and maximum) were calculated for each gender-age class. "Adults" from PHR were compared to "adults" from FFS. The comparison was restricted to "adults" since the numbers of "weanlings" and "juveniles" were small on all three islands. In addition, the small number of seal samples collected at MID prohibited a meaningful comparison of this island with the other two.

Mean differences were calculated, their statistical significance determined via two-sample *t*-tests, and their 95% confidence intervals were constructed. When an F-test indicated that a variable's population variances were different on the two islands, the two-sample *t*-test and the 95% confidence interval were calculated based on this assumption. Otherwise, equal variances were assumed in the calculations. Since some of the variables appeared to be non-normal, the non-parametric Wilcoxon Rank Sum Test was performed and its *p*-value was compared to the corresponding *t*-test *p*-value. Comparisons between islands were performed separately for male and female seals. For variables where results for males and females were similar, analyses were repeated for males and females combined to increase power and obtain narrower 95% confidence intervals. However, combined analyses were not conducted for variables where the mean differences between the islands differed considerably for males and females and for variables where the mean difference was near zero for both males and females. In these situations, the combined analyses did not add important information and could have obscured differences which were seen in the gender-specific analyses (for example, the mean value was significantly different among males, but not among females, and the combined analysis would not be expected to be informative).

The sample size for the serologic study was determined by assuming (1) that the detection of a disease process is a binomial process yielding positive (present) or negative (absent) results; (2) by setting desired power of the laboratory analyses at 80% and minimum number of successful trials at five; and, (3) by assuming that an endemic disease found in a subpopulation would be present in at least 20% of the seals. The small sample size at MID reflects 20% of the entire subpopulation which is estimated at 45 to 50 seals.

RESULTS

The first portion of this analysis constitutes an initial health assessment to determine whether individual morphometric, hematological, or biochemical parameters differ on the two islands which are characterized by differences in their population dynamics. Findings from this analysis, in turn, may be used to generate specific hypotheses regarding medical conditions that may be playing a role in the success of maintenance of population size. If medical hypotheses are developed from these screening data, they may be tested with more targeted approaches to identify specific conditions which are impacting health.

Seals

A total of 107 monk seals from both sexes (37 females, 64 males, six unknown) and different age classes including weaned pups (12), non-adults (37), adults (52), and six of unknown size were sampled. Sample size distribution by site includes 51 seals at FFS, 46 seals at PHR, and 10 seals at MID (Table 2). Monk seals were captured for several reasons and, in some cases, opportunistic sampling was performed. A total of 71 seals were specifically sampled for this epidemiological survey. Twenty-six seals were captured at FFS (8) and PHR (18) for satellite radio deployments and retrievals, and 10 seals at FFS were captured for critter cam attachments and retrievals. Several seals were sampled twice during these activities. Seals were sampled during several periods of time depending on the activity or opportunity for sampling (i.e., critter cams, satellite tagging, epidemiologic studies). At FFS, samples were collected on March 9-21, 1997, June 30-August 23, 1997, May 25, 1998, June 7, 1998, and July 7-August 9, 1998. At PHR, seals were sampled during October 25-November 11, 1997 and February 16-22, 1998. Seals at MID were sampled June 25-29, 1998 .

Clinical Exams

Following physical restraint with a hoop net, monk seals (n = 106) were tranquilized with diazepam at approximate doses ranging 0.05 to 0.46 mg/kg. All monk seals included in this study were considered healthy and had normal body condition except for a very thin seal at MID. This seal was reported dead on August 1998, possibly from a shark attack. All clinical parameters were similar between adult and non-adult seals. No significant abnormalities were noted on any animal except for old scars and marks that were used to identify individuals. One adult seal at PHR presented fresh wounds caused by a cookie-cutter shark (*Isistius brasiliensis*) but no clinical problems were observed. External parasites were not found on any of the monk seals examined.

Morphometrics

Descriptive statistics for the gender distribution of adult seals on FFS and PHR are shown in Table 2. On FFS, roughly one-third of the adults were female, whereas females comprised approximately half of the total adult populations. Statistics for morphometric variables were also summarized (Table 3). As expected, average size and mass increased with age. Female weanlings were generally larger than males; their girth and mass was significantly greater (P = 0.02-0.03). However, the girth and mass of juvenile males exceeded that for females; the difference in girth was statistically significant ($P = 0.02$). Adult females were longer, had greater girth, and weighed more than adult males; all differences were statistically significant at $P = 0.01$ or less. For all three variables standard deviations were large relative to the mean (especially among juveniles and adults) and thus, the variables range was wide. Morphometric parameters were compared between adults at FFS ($n = 34$) and PHR ($n = 29$).

The mean differences of selected continuous variables between adults at FFS and PHR, the p-values of the two-sample t-test testing for the significance of the differences, and the 95% confidence intervals for the difference between the two means are presented in Table 4. Variables that have extremely non-normal distributions are not included in this analysis. It must be kept in mind that, due to the large number of tests, some of the results may be significant due to chance. The difference in gender distribution between the two subpopulations being compared and the finding from the descriptive analyses presented above that some variables differ across genders (e.g., mass) dictate a gender-specific approach in examining interpopulation differences for these variables. Among female seals, no statistically significant differences among islands with respect to the morphometric variables were found. However, PHR females tended to be longer, have greater girth, and to weigh more than FFS female adult monk seals. Male seals on FFS showed the same tendency for having smaller girths and weighing less than males on PHR, although none of the differences were statistically significant. The average mass difference is approximately 6 kg (12.5 lb) for females and 5 kg (10.5 lb) for males. The difference is observed in estimates of length, girth, and mass. Overall, PHR females and males were heavier than their respective gender groups at FFS. This difference was present despite the fact that the average ages of male and female adults were between 10 and 11 on both islands. When males and females were combined, the morphometric variables were still nonsignificant. However, we found a large positive average mass difference between seals on the two islands with a t-test p-value close to 0.1, and the center of the 95% confidence interval much greater than 0. Since the mean difference for the combined group was similar to the differences found for males and females separately, it appears that this finding was not artificially produced by gender differences on the islands, but that seals at FFS did tend to weigh less than seals on PHR.

Clinical Pathology

The results for the hematological variables are shown in Table 5. There were too few female weanlings to provide a valid statistical analysis for comparison. For the older animals the means of the hematology variables were similar for females and males. Averages were also

similar for juveniles and adults. However, the mean of the percentage of eosinophils was much smaller among weanlings than juveniles and adults (result based on small numbers). Table 5 also includes the descriptive statistics for the biochemistry variables. Most notable is the great variability of the observations for most variables as demonstrated by the large standard deviations compared to the means for each parameter.

Adult female seals at FFS had higher mean white blood cell counts than did females on PHR ($P = 0.05$). However, none of the components of the differential count nor the packed cell volume was different among female seals from the two subpopulations. Among adult males, the mean white blood cell counts were similar for FFS and PHR. However, statistically significant increases were found for percent neutrophils at FFS, accompanied by a decrease in the percent lymphocytes and eosinophils when compared to PHR. Since the percentages of bands and basophils were in the normal range for all adults on both islands, and were not normally distributed, no comparisons between the two islands were made. The findings for male and female monk seals collectively, which include evidence of increases in white blood cell count and percent neutrophils and a decrease in percent lymphocytes deserve attention. Although the changes affect different WBC parameters within genders of monk seals, these findings are compatible with a subclinical disease process affecting FFS monk seals.

Significant differences were found for a number of biochemistry variables (Table 6). Albumin, creatinine, cholesterol, glucose, and Na/K ratio averages were significantly higher among females on FFS ($P = 0.05$); conversely, GGT, LDH, BUN, potassium, and B/C ratio averages were significantly lower for females on FFS. At the 0.1 level of significance the bicarbonate average was greater and the chloride average was smaller on FFS. Large mean differences were also seen for SGPT and CPK. However, the standard deviations of these variables were very large resulting in a loss of power. Even though the respective 95% confidence intervals included 0, their centers were considerably greater than 0, and it, therefore, seems likely that SGPT and CPK levels tend to be considerably lower on FFS. Collectively, it is difficult to find a coherent pattern in these results. For example, for BUN and creatinine, both tests of kidney function are significantly different, but in opposite directions among female monk seals.

As was the case for females, the averages of the biochemistry variables albumin, creatinine, and Na/K ratio were significantly higher for males from FFS (borderline significance for albumin), and the averages of BUN, potassium, and B/C ratio were significantly lower. However, no significant difference between the islands was found for glucose, LDH, and GGT (although the *p*-value of the *t*-test for GGT was near 0.1 and the center of the 95% confidence interval was much different from 0). Interestingly, contradictory to the result for females, the cholesterol average of males was lower at FFS than at PHR. However, this result was not statistically significant. Unlike the findings for female monk seals, among males the average calcium level was significantly lower on FFS, and the mean difference for AP was very large and significant ($P = 0.1$; center 95% confidence interval > 0). No significant mean difference was found for CPK.

For the hematology variables, the only variables with similar results for males and females (Tables 7 and 8) were the percentage of monocytes and the packed cell volume. The mean differences of both variables were still nonsignificant in the combined group. Among the biochemistry variables, average differences for albumin, creatinine, calcium, potassium, and Na/K ratio were still significant and their 95% confidence intervals were narrower than the confidence intervals obtained from the separate analyses of males and females (Tables 7 and 8). Mean differences of BUN and B/C ratio were also still significant in the combined group. However, since the mean difference for both variables for males was much higher than for females, the mean difference in the combined group fell between the two values, and the 95% confidence intervals, though narrower, were shifted compared to the 95% confidence intervals obtained in the separate analyses (Table 9). The mean differences for protein and anion gap were still nonsignificant. No other variables were considered since the mean differences of the remaining variables either differed considerably for males and females or were near zero for both males and females (Table 9).

In cases where the normality of a variable was questionable, the significance of the mean differences was tested using the nonparametric Wilcoxon Rank Sum test, and the resulting *p*-values were compared to the respective *t*-test *p*-values. Since conclusions regarding the significance of the mean differences did not change for any variables, the nonparametric results are not presented. Only the Wilcoxon Rank Sum test results for SGOT deserves mention. The median SGOT for males on FFS was 73 compared to a median of 91 for males on PHR. A similar but less pronounced trend was seen for females. When males and females were combined, the Wilcoxon Rank Sum test *p*-value of 0.066 was borderline significant (Table 9).

Atrial Natriuretic Factor

Results of sera tested from 41 seals from PHR for circulating ANP (proANF 31-67) yielded mean values of 91.8 ± 9.6 (10-235) pg/ml. Five of the seals presented values above 200 pg/ml for proANF 31-67 (i.e., vessel dilator). The circulating ANP detected in monk seals was one-third to one-half lower than those found in humans. The majority of the ANP values were in the low end of the radioimmunoassay curve. Samples from FFS are pending.

Serology

This component of the health assessment considers infectious disease agents which may be prevalent in HMS and is the first comprehensive effort to screen HMS subpopulations in the wild for these agents. Serum specimens from 125 seals (64 at FFS, 10 at MID and 51 at PHR) have been tested to selected infectious agents known to be present in marine mammals (Table 1).

Morbillivirus

The sera were tested by differential serum neutralization for antibody to canine distemper virus (CDV), phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV). Three seals were found to have antibody to either DMV, PMV, or both antigens. These three positive sera were contained in the batch of 44 samples collected at FFS between June 8 and July 27, 1998, with the exception of two samples obtained in May and June of 1998. No antibody to morbillivirus was found in six samples collected on FFS in March 1997, nor in 14 samples collected between June 30 and August 23, 1997 from FFS. Therefore, the prevalence of antibody was 0% (0/20) in 1997, 6.8% (3/44) in 1998, and 0% (0/46) in 1999 on FFS. There was no antibody detected to morbillivirus in 10 HMS obtained on MID in 1998, nor in 26 samples from HMS obtained in PHR in 1997, nor in 25 samples from the same site collected in 1998. Low antibody titers were detected in a 2-year old female from Little Gin, a 12-year old female from Tern, and an adult male from Tern. One seal presented antibodies to PMV alone; one to DMV alone; and one to both PMV and DMV. The highest titer was found to PMV (1:16) during the first testing performed in August 1998. Positive samples were retested blindly with the highest titer found again for PMV at 1:48. Testing of 80 serum samples for CDV and PDV at FADDL reported negative results; however, samples were not tested for PMV or DMV.

Thus, if confirmed, antibody would have been present in HMS from two of the six locations where sampling occurred, and not restricted to a single age class or gender. Due to the potential implications of this finding, further serological testing of HMS was conducted during January 13 to February 4, 1999. A total of 46 additional HMS samples were obtained, including two of the three animals found to be positive earlier. All sera were found negative at titers less than 1:8 for CDV, PDV, DMV, and PMV.

Brucella

During the 1997-98 screening of HMS populations, 5 of 51 (10%) seals at FFS, 4 of 10 (40%) seals at MID and 9 of 46 (20%) seals at PHR were positive to one or more tests for the detection of antibodies against *Brucella abortus*. Thus, the overall prevalence of antibody to *Brucella* was 19/107 or 17.8%. Both age and sex classes were represented among the seropositives. An adult female captured at PHR during October 1997 and February 1998 tested positive on both occasions. Results reported varied depending on the testing laboratory (Table 10). All specimens were negative for antibody to *Brucella canis*.

The significance of this antibody is unknown at this time. Table 9 provides data for *Brucella* seropositivity from three laboratories, FADDL-Plum Island, USDA, OADDL-Oklahoma Animal Disease Diagnostic Laboratory, and NVSL-National Veterinary Services Laboratory, USDA. Serious discrepancies exist in the findings for antibody against *Brucella abortus*, using standard tests developed for the control and eradication of bovine brucellosis by USDA. Among 19 HMS for which at least one laboratory reports the sera as positive, there is a confirmatory positive report from the second laboratory in only 9 (47%) of the animals; there are

8 animals in which Oklahoma reports a positive and Plum Island a negative, and two in which Plum Island reports a positive, but Oklahoma finds the sample negative. Two of the animals in Table 9 were sampled twice (BC05, R2AG). R2AG is positive in both labs each time; BC05 is negative at Plum Island and positive at Oklahoma on the first sample and then positive in both laboratories 4 months later. The use of the screening card test to denote positivity and negativity by the Oklahoma Laboratory accounts for much of the variability between laboratories.

In summary, serologic testing for *Brucella* conducted in HMS to date has been inconclusive, despite the use of standard techniques in our national reference laboratories. Although the evidence suggests that *Brucella* strains may be circulating in HMS, the extent of this infection is difficult to determine because of conflicting findings between laboratories. To date, testing has only been done for antibody to *Brucella abortus;* there have been no attempts to develop a serologic assay for the organisms isolated from marine mammals.

Other Antigens

The serum samples collected from PHR that were positive to walrus adenovirus were BP33, BC51, BC39, and R2AG with ELISA corrected optical density (OD) readings of .206, .393, .258, and .223, respectively. Samples collected at FFS were considered suspect positive to this virus (YL61 and YJ05, readings .153 and .169, respectively). One sample from PHR (BC51, ELISA reading .206) was positive to walrus retrovirus-like agent. BP05 was considered suspect with an ELISA reading of .198. In addition, two samples (YL73 and Y758, readings .277 and .449, respectively) were positive to marine herpesvirus 206. Sample YU29 collected at FFS had high titers (ELISA reading 1.074) to human herpesvirus II. Two samples (YZ06 and YP66, readings .188 and .142) were considered suspect. It is important to emphasize that these suspect positives (below 0.200) presented considerably higher values than the OD readings for most samples.

Positive titers to *Chlamydia psittaci* using the complement fixation test were identified in 40 of 44 (91%) seals tested at FFS, nine of nine (100%) seals tested at MID and 25 of 26 (96%) seals tested at PHR. A seal in FFS was tested twice (Y758). Seals of both sexes and all age classes were represented in the positive samples. Positive titers of 25-500 to *Toxoplasma gondii* were identified in two adult females at FFS (YL73) and MID (RF06) using the MAT. In addition, 5 of 51 (10%) seal specimens collected at FFS presented weak positive reactions to the ELISA *D. immitis* antigen test. Seals included two adult males (YN82 and YL22), two male weaners (YY30 and YY39), and a subadult female (YJ05). Serum specimens from YL22 were tested during two different occasions with similar results.

All 120 specimens were serologically negative to caliciviruses (32 serotypes), California sea lion rotavirus, walrus enterovirus 7-19, CDV, PDV, seal herpesvirus 1, vesicular exanthema of swine (12 serotypes), seal influenza virus, and *Leptospira pomona* (+5 serovars).

Virology

A total of 64 buffy coats from heparin tubes, 47 buffy coats from EDTA tubes, 5 buffy coats from tubes without anticoagulant identified, and 70 rectal swabs were processed for virus isolation, and all were passaged three times (1 week per pass) on Vero cells and PK_{15} cells. No cytopathology was observed and no virus isolations occurred during the three weekly passages. Samples collected at FFS (YU29, YY13, YY30, and YY33), PHR (BP03, BC39, BZAG, BP05, B5AY, BX40, BCRN, BX04, and Y529), and MID (YZ03) were positive for caliciviral presence using the specific group epitope binding monoclonal antibody. Four of the strongly positive rectal samples to calicivirus (YU29 and YY13 from FFS, and BP03 and BX04 from PHR) were examined by negative stain electron microscopy and virus particles morphologically identified as caliciviruses were observed. All second and third passage materials were negative by dot blot, indicating that the calicivirus present in these rectal swabs was not replicating in vitro (not growing in Vero or PK_{15} cells).

Nine of the rectal swabs from seals at PHR and one from MID that were further processed for caliciviral presence were positive ranging from 1+ to 4+ reaction intensity. Two seal specimens from PHR (BP03 and BX04) were further processed for direct electron microscopy to confirm the specificity of the dot-blot reactions and virus-like particles morphologically identified as caliciviruses; however, second and third passage materials were also negative.

Bacteriology

A total of 62 rectal swabs were collected for enterobacterial isolation including 26 of 35 (74%) from FFS, 3/11 (27%) from MID, and 7/26 (27%) from PHR. Three bacterial groups were isolated. *Edwardsiella tarda* was isolated from eight seals at FFS, two at MID, and seven at PHR. *Escherichia coli* was isolated from 12 seals at FFS. Most *Salmonella* isolates originated from weaned seals sampled at FFS, except for an isolate of *Salmonella* group E from an adult female, and *Salmonella* group B isolated another adult female from PHR (Table 11). It is important to note that no weaned seals were sampled at other sites.

Parasitology

A total of 39 PVA-fixed fecal specimens were examined at FFS ($n = 10$), MID ($n = 10$), and PHR ($n = 19$). Overall, 35 of 39 (89.7%) of PVA-fecal specimens were positive for ova of one or more helminth species: 87% were positive for *Diphyllobothrium* ova; 38.5% were positive for both *Contracaecum* and *Dipyllobothrium*; and 2 (5%) were positive for *Contracaecum*, *Diphyllobothrium*, and acanthocephalan ova. Acanthocephalan ova were found in five samples (12.8%) (Table 12). The species mix was similar for specimens from PHR, MID, and FFS except that the prevalence (100%) and intensity (mean, 2,011 ova per smear) of *Diphyllobothrium* was higher in specimens from PHR. The findings for *Contracaecum* are

similar to the prevalence and intensity of infections found in Hawaiian monk seal scat specimens collected in recent years (Kliks, unpubl. data). Tapeworm egg densities were higher at PHR averaging 2,469 ± 1,027 (65-10,980) eggs/slide than in MID at 912 ± 478 (0-4,760) eggs per slide. Ascaridoid ova were also more common in PHR at 7 ± 3 (0-24) eggs/slide when compared to MID at 3 ± 2 (0-12) eggs/slide. No protozoan cysts or trophozoites were noted in the 2 unfixed and 39 PVA fixed fecal specimens examined directly and all 26 Hematoxylin-stained PVA fecal films.

Toxicology

This final component of the health assessment was conducted to establish the body burdens of chlorinated hydrocarbons, including pesticides and PCBs and to determine whether differences in concentrations of these contaminants exist across subpopulations of HMS. Analysis of chlorinated hydrocarbons (CHs) were completed in 53 blood and 11 blubber samples. All blubber samples were obtained from adult males over 5 years old. Relatively low concentrations of CHs were measured in tissues, especially blood as compared to blubber. It was found that mean lipid concentration (lipid 41%, $n = 6$) in monk seal blubber was approximately 400 times higher than the mean lipid level in corresponding blood (lipid 0.11%, $n = 6$). Preliminary statistical analyses compared the log transformed summed chlorobiphenyl and summed DDT concentrations at the three sites. Based on wet mass, significantly higher ($P \leq 0.05$) total CB concentrations were found in blood from seals sampled at MID (7.8 ± 3.8 ng/g) compared to blood at FFS (4.4 ± 3.3 ng/g) and PHR (3.5 ± 1.2 ng/g). The same trends were observed for DDT levels.

The predominant CHs measured in monk seal tissues were moderately chlorinated CB congeners (containing 5-7 chlorine atoms). The most abundant CB from MID was CB 153. The dioxin-like CBs were comprised primarily of mono-*ortho* (118 and 105) and di-*ortho* substituted (180) congeners.

DISCUSSION

The results of this study provided standardized health and disease information from subpopulations of Hawaiian monk seals living in the wild for the first time. This represents the most comprehensive effort to date to establish baseline values for a number of parameters for the endangered Hawaiian monk seal. This information will form the basis of the biomedical decision process for future translocation of individual seals among subpopulations. The work described herein is part of a larger effort to establish methods to conduct comprehensive health assessment of Hawaiian monk seal populations in order to monitor disease status (Aguirre et al., 1999).

Clinically, individual seals at all subpopulations were considered normal and healthy with one exception. The morphometric data suggest that adult seals of the same age from FFS tend to be smaller than those on PHR. The differences in morphometry between adults of both genders are consistent with field data reporting that the monk seal population on PHR is experiencing better growth and maintenance than the population on FFS and suggests that the approach taken in this analysis of health parameters has merit.

A previous report (Banish and Gilmartin, 1988) examined these parameters in 12 recently weaned monk seals that were captured in the wild and held in captivity. More recently, an analysis of hematologic and serum biochemistry values was conducted for 10 captive female seals brought also as weanlings to Oahu and held in tanks after capture in the wild and sampled every 6 to 8 weeks over the course of 4 years (NMFS, 1995-99, unpubl. data). The present analysis was performed with age and gender stratified data in order to establish baseline normal values for each class of wild seals. In general, the data for these parameters in free-ranging Hawaiian monk seals fell within the ranges of values previously described for the comparable age classes for weanlings and juveniles. They are also generally comparable to ranges for other adult pinnipeds such as the elephant seal (*Mirounga angustirostris*), to which the monk seal could be compared (Dierauf, 1990; Castellini et al., 1993; Rea et al., 1998).

In the hematological data, there is evidence for increased numbers of circulating white blood cells in females from FFS, and for increases in neutrophils and decreases in lymphocytes among males. These data reflect the possibility of the presence of a subclinical disease. Eosinophil differences between weanlings and other size classes may reflect the acquisition of parasite burdens and a subsequent hematologic response. Standard deviations were generally large relative to the mean, reflecting the great variability of the data. However, the lack of an elevation in total globulins in seals from FFS speaks against the hypothesis that a low grade chronic infectious disease process is affecting monk seals on FFS.

In reviewing the blood smears from the free-ranging seals, a number of interesting features are worth describing including the presence of large numbers of macroplatelets (with a general high variability in platelet size), high absolute numbers of eosinophils, and the presence of occasional atypical and reactive appearing lymphocytes. Mean platelet volume (MPV) in other species has been used to assess bone marrow response to thrombocytopenia or indicate a regenerative response to thrombocytopenia. Given the fact that platelet numbers were considered adequate in all animals sampled, the presence of macroplatelets in free-ranging monk seals may not be related to a regenerative response. However, it may indicate other conditions such as splenic contraction secondary to epinephrine mediated stress during capture with subsequent release of these large platelets. Baseline MPV values as determined for captive Hawaiian monk seals may provide valuable information in the evaluation of future medical problems or in the assessment of stress in free-ranging seals. It is recommended that if whole blood from free-ranging seals is submitted to a reference laboratory, the determination of MPV should be requested.

In free-ranging seals, the absolute eosinophil counts ranged from 0-2400 cells/ _l, corresponding to 0-26% of the total leukocyte number. Captive monk seals rarely had absolute eosinophil counts greater than 500 cells/ _l. Eosinophil counts are likely increased in free-ranging animals secondary to an increased endoparasite burden. Usually, peripheral eosinophilias are elicited with parasites that have a long-term and intimate association with the host (i.e., nematodes with extensive migration patterns or intestinal adhesion as well as lungworms and heartworms).

The reactive and occasional atypical lymphocytes noted showed one or more of the following morphologic attributes: atypical nuclear morphology (especially cleaved or indented, rarely cloverleaf shaped nuclei), cytoplasmic basophilia, increased cell size, rare nucleolar remnant, and occasional azurophilic cytoplasmic granulation. These changes may be considered nonspecific and may be related to lymphocyte stimulation secondary to environmental antigenic exposure. In other mammals, these changes can be noted post vaccine administration, in allergic and inflammatory skin diseases, with numerous systemic infectious diseases such as *Ehrlichia*, and with inflammatory diseases of the intestinal system, among other causes (Duncan et al., 1994).

Regarding the analyses of biochemical parameters which were concordant across species for the interisland comparisons, increases in albumin, creatinine, and Na/K ratios were observed on FFS, while BUN, potassium, and chloride concentrations were lower on FFS among both female and male monk seals. As stated above, conflicting results in tests of renal function make these observations difficult to interpret. Perturbations in concentrations of plasma electrolytes (Na, K, Cl) in monk seals on FFS merit further consideration of their potential clinical implications. The significance of differences that fall within "normal" ranges for these parameters is difficult to interpret.

LDH values reported herein for wild Hawaiian monk seals correlate to values reported in other mammals experiencing a stressful situation. Elevations in liver and muscle associated enzymes are common findings with capture related stress (hypoxia) and myopathy in free-ranging animals (Williams and Thorne, 1996). The increase in globulins observed in wild seals is compatible with the finding of low to moderate numbers of reactive and atypical lymphocytes noted in the blood smears.

Atrial natriuretic factor (ANF) is a hormone that is synthesized by and secreted from the heart. The most effective and best documented stimulus for ANF release is atrial distension and it appears that this stimulus is universal amongst the vertebrate groups. If present in the serum of monk seals, it is most likely that ANF has a key role in the regulation of blood pressure and could serve as another indicator of individual health.

Preliminary serologic results in Hawaiian monk seals have determined that several potential pathogenic agents are circulating at these three subpopulations. The significance of the low titers to DMV and PMV identified in three seal specimens at FFS was initially unclear. They probably represent "false positives" due to laboratory error. However, the possibility remains

probably represent "false positives" due to laboratory error. However, the possibility remains that the antibody detected may represent cross-reaction with serologically related viruses. Osterhaus et al. (1998) recently described isolation and partial characterization of a morbillivirus obtained from three Mediterranean monk seals (*Monachus monachus*) which died during an outbreak of unknown etiology that killed 300 seals on the western Saharan coast of Africa and a second from a Mediterranean monk seal from Greece. The former isolate was most closely related to DMV and the latter to PMV.

Infection with morbilliviruses is considered to hold the most devastating potential impact for the long-term survival of the Hawaiian monk seal. Introduction of a morbillivirus to a naive population of monk seals could be catastrophic for the recipient population during translocation efforts, and therefore, for the species as a whole. Antibodies to PDV were not detected in serum collected from 80 Pacific harbor seals along the northwest coast during 1992-93 (Duignan et al., 1995). However, serological evidence of morbillivirus infection was recently found in small cetaceans from the Southeast Pacific, thus providing a potential pathway for transmission to the Hawaiian monk seal (Van Bressem, 1998). More recently, 6 of 18 stranded common dolphins (*Delphinus delphis*) in southern California tested antibody positive for DMV, and morbilliviral RNA was detected in three of these five by RT-PCR (Reidarson et al., 1998). This represents the first report of morbillivirus infection in the northern hemisphere of the Pacific Ocean. The possibility of dolphin-to-seal contact and transferring of infection cannot be discarded. Therefore, continuing vigilance for infection of HMS with morbilliviruses is strongly recommended.

The risk of morbillivirus infection justifies the need for continuous screening and raises the possibility of developing preventive measures such as vaccination. During the summer of 1991, four captive Hawaiian monk seal males, housed at the Waikiki Aquarium, were vaccinated with a CDV-ISCOM vaccine as described in previous vaccination studies (Visser et al., 1992). Blood samples were collected at different days post-inoculation and shipped for testing to A. Osterhaus (Erasmus University, Rotterdam, Netherlands). By using a CVD-ELISA, the titers recovered ranged from < 20 to 60. These titers were low even using the booster vaccination that is known to increase titers in harbor seals. Due to the fact that no virus neutralization results were available at the time and that no information was available on the applicability of protein A for detecting Hawaiian monk seal immunoglobulins, the data is difficult to interpret (A. Osterhaus, unpubl. data, 1997). Further studies including the use of DMV and PMV antigens may be required depending on future field serologic results.

The lack of group antibodies to caliciviruses in the specimens tested is inconsistent with previous studies (Poet et al., 1993). Caliciviruses were detected in this study using a monoclonal antibody probe and direct electron microscopy but could not be cultured in vitro from rectal swabs. A report previously published (Poet et al., 1993) confirmed the presence of caliciviruses in both avian and fish specimens collected at FFS. It is interesting to note that the seal YZ03 positive to calicivirus recently sampled at MID was a seal born and collected at FFS in 1991 and brought to Oahu for rehabilitation. She was released at MID in 1992 and has been sighted there consistently ever since.

Antibody to the phocine herpesviruses appears to be widely distributed; antibodies to PHV-1 have been detected in the Antarctic from Weddell seals (*Leptontchotes weddelli*) and in the Arctic from harp and hooded seals (*Cystophora cristata*) (Gulland et al., 1997). In a recent study conducted in Alaska, antibody to PHV-1 was found in 29-77% and to PHV-2 in 16 to 50% of a sample of pinnipeds collected from 1978 to 1994 (Zarnke, 1997). During a stranding event of 700 live Pacific harbor seal pups, 54% died. Seals examined had lesions suggestive of a herpesvirus. A herpesvirus-like virus was isolated from adrenal tissue on cell culture (Gulland et al., 1997).

It is recommended that diseases such as the ocular syndrome, developed in the captive seals, are carefully monitored in the wild. Future testing for this disease and other important infectious diseases in Hawaiian monk seals destined for translocation should be aggressively pursued which was previously recommended (Aguirre et al., 1999). The continuous development of cell lines, immunoglobulins, and reagents specifically for monk seal testing (Lu et al., 1998) will provide a better understanding of the immunologic and virologic status of the species.

Results from this study indicated that 12-40% of the studied subpopulations presented were suspect to positive titers to a *Brucella abortus*-like organism. Brucellosis is an important infectious disease of many mammalian species including humans. Infection is typically followed by abortion or stillbirth and by epididymitis and infertility in males. The disease is spread horizontally by contact with infective discharges from aborting females, by ingestion, and by other routes. The genus *Brucella* has been recently identified in marine mammals isolated from common seals (*Phoca vitulina*), a grey seal (*Halichoerus grypus*), a hooded seal (*Cstophora cristata*), and several species of cetaceans (Ross, 1996). A similar *Brucella* species was isolated from the aborted fetus of a bottlenose dolphin along the California coast. The strains do not appear to be members of known species of the organism and a new species has been proposed (Jahans, 1997). Recently, *Brucella* titers were detected in 18 of 102 Pacific harbor seals and 4 of 50 California sea lions from Puget Sound, Washington indicating relatively widespread infection among Pacific coast pinnipeds. The organism was recently isolated from *Parafilaroides* lungworms in a Pacific harbor seal suggesting a potential role for the parasite as a secondary mechanism of transmission (Garner, 1997). Further studies should include the collection of tissues from necropsied seals to isolate, culture, and further describe the nature of brucellosis in monk seals. It is recommended that Hawaiian monk seals with antibody to *Brucella* not be considered candidates for translocation (Aguirre et al., 1999).

Leptospira antibodies have been previously reported in two juvenile monk seals tested during translocation efforts at FFS in 1992. Leptospirosis is a zoonotic disease affecting a wide range of hosts. All isolates from pinnipeds have been typed as *L. i. pomona* which is a pig-adapted serovar. Pinniped to human transmission has been demonstrated. Leptospirosis has not been documented in phocids until recently (Gulland, 1999). Continuous testing is recommended. Seals with positive titers are not suitable candidates for translocation.

The high prevalence of antibodies to *Chlamydia psittaci* (91-100%) in all three subpopulations is inexplicable. The CF test used is not a reliable indicator of individual animal infection as reported by others (Booth and Blanshard, 1999). Chlamydiosis is known to cause reproductive tract disease in other mammals and may cause infertility. Further testing of monk seals should include cell culture, DNA, and PCR attempts from vaginal and penile urethra epithelial swabs coupled with the antigen ELISA such as the Surecell system (Eastman Kodak Co.). Chlamydial infection and antigen presence have been reported from the marine environment in sea turtles (Aguirre et al. 1994). Close long-term monitoring for both *Brucella* and *Chlamydia* may shed some light on the decline of monk seals at FFS.

All weaned pups sampled at FFS yielded *Salmonella* spp. In comparison, other age classes are not infected with the exception of an adult and a juvenile seal sampled at FFS and PHR (Table 9). According to records archived at NMFS Honolulu Laboratory, 67% of seals brought for rehabilitation purposes to Oahu between 1989 and 1995, and sampled upon arrival or during the rehabilitation process, were positive by rectal swabbing and culture to one or more serotypes of *Salmonella*. Serotypes isolated included *S. bredeney, S. give, S. havana* (the most common isolate), *S. johannesburg, S. minnesota, S. oranienburg, S. reading, S. san diego,* among other serotypes not identified. Serotypes identified during the present study included *S. cerro, S. chester, S. minnesota, S. oranienburg* and *S. san diego*. No *S. havana* was identified.

To date, a question remains whether *Salmonella* infections are endemic to the monk seal population and their clinical significance in the population. Salmonellae were suggested as a cause of disease in an earlier survey of 19 immature seals to be translocated from FFS to MID in 1992. Salmonellae were isolated from several seals. Two of seven seals died of bacterial infection and aspiration pneumonia during their rehabilitation at Oahu. The role of *Salmonella* infection in the deaths was unclear, but it is likely that it represented a contributing cause of mortality (Gilmartin et al., 1993; Poet et al., 1993).

The absence of clinical signs and the high prevalence in weanling seals are not surprising. Gilmartin et al. (1979) reported a high prevalence of this potential pathogen in a pinniped population as rookeries are heavily contaminated with fecal material. The weaned seals sampled during this study were within few meters of each other despite the fact that monk seals are sedentary animals during most of their life history as compared to other pinniped species. Another source of infection could be explained by the presence of thousands of seabirds and the their close contact with seals. It is recommended that surveys for enterobacterial pathogens and Salmonellae continue at other sites and samples should include weaned seals. Close monitoring of *Salmonella* in seals identified for translocation is necessary since periods of stress, such as confinement, rehabilitation, and translocation may allow *Salmonella* spp. to become pathogenic (Thornton et al., 1998). The organism has been associated with mortality in monk seal pups undergoing rehabilitation in captive settings. This may pose a difficult problem due to the combination of high prevalence and pathogenicity for this age class of seals and make it difficult to find suitable candidates for translocation. Horizontal transmission of salmonellae during holding periods in shoreline pens presents a second problem for which further evaluation is required. Further work defining the salmonella infection status of each of the outlying islands at

different age classes is recommended to establish the endemicity of the agent.

Fatal toxoplasmosis caused by the coccidian protozoan *Toxoplasma gondii* has been commonly diagnosed in marine mammals. The condition has been identified in the Hawaiian Islands from a stranded spinner dolphin (*Stenella longirsotris*) and a captive California sea lion (*Zalophus californianus*). The source of infection for wild animals remains unknown but ingestion of animal carcasses and sea birds has been suggested (Dubey, 1987). The clinical cases in marine mammals appear to be incidental (Oksanen et al., 1998). During this study, two seals from FFS and MID presented antibodies to *T. gondii*. At present, there is no reason to believe that toxoplasmosis may represent a health problem for Hawaiian monk seals during translocation efforts. Weak reactions to heartworm antigen test requires further investigation. All seals originated from FFS, a population characterized for heavy parasite burdens. A possibility is that these weak seropositives represent cross-reactions to other parasite antibodies. Dirofilariasis in domestic canids is endemic in the main Hawaiian Islands.

The significance of titers identified in a few seals at FFS to walrus adenovirus, walrus retrovirus-like agent, marine herpesvirus, and human herpesvirus II is unclear. Continuous surveillance paired with virus isolation attempts may provide a long-term pattern of these and other infectious agents in the population. Larger sample sizes and continuous monitoring may answer some of these questions regarding exposure to marine viruses and corroborate the possibility of emerging diseases in the population.

The endoparasites identified in this preliminary study have been previously reported in Hawaiian monk seals (Golvan, 1959; Rausch, 1969; Dailey et al., 1988). All parasites were present at all subpopulations, therefore concerns should be focused on the intensity of parasitism of individual seals prior to their translocation. Little is known about the direct effects of massive infection with *Diphyllobothrium* species common in this host. Similar *Diphyllobothrium* species in humans and other animals are known to cause considerable morbidity. The gastric ascaroid, *Contracaecum* is a large and robust anisakid nematode worm. Infection with even one larval form of this and related species causes severe acute gastrointestinal distress (Whittow and Balazs, 1979). The present study indicates a high prevalence and intensity of infection at FFS. Further pathologic studies are necessary to determine if *Contracaecum* infections cause serious morbidity in both adult and immature monk seals. An overall interpretation of the impact of helminth infections on the Hawaiian monk seal includes the evaluation of fresh and preserved specimens of organ systems and tissues.

Final results of toxicologic specimens and proper statistical analysis comparing size and sex variables will provide a better understanding of the contaminant levels. Analysis of more samples is warranted to better establish the apparent differential accumulations of CHs in tissues of monk seals at MID compared to the other two subpopulations. Although these preliminary data suggest that levels of certain organic contaminants are not elevated compared to other pinniped species, the highly endangered status of Hawaiian monk seals support caution in evaluation of the level of risk posed by toxic chemicals. Future testing should include endocrine disruptors, heavy metals, and additional analytes.

CONCLUSIONS

This study provides the first systematic description on the health and infectious disease status of wild Hawaiian monk seals. Baseline values for hematology, serum biochemistries, serology, virology, bacteriology, parasitology, and toxicology provided important information necessary for translocation efforts of this endangered species. Overall, the analysis of simple markers of health status for monk seals in two subpopulations characterized by differences in population dynamics show that there is good potential to use such an approach as a tool for health assessment. The evaluation of clinical significance of some of these findings merits investigation. Based on this study, our strongest tool to avoid the translocation of infectious agents remains to be serologic testing. The findings above lend credence to the concept of health assessment described in the Hawaiian Monk Seal Epidemiology Plan (Aguirre et al., 1999). It is recommended that plans should be developed for continuous surveillance of these and other subpopulations to confirm observed trends and to follow-up studies to test specific hypotheses using more sophisticated techniques.

There were no findings in this study that serve as a contraindication to translocation as a strategy to enhance population recovery. Based on the findings of this health assessment, the procedures recommended for translocation in the Epidemiology Plan (Aguirre et al., 1999) appear to be sound. The performance of serological testing prior to translocation should be maintained as a condition for movement of HMS between islands. In particular, the agents identified as tier one in the Epidemiology Plan must be tested for. The finding of possible exposure to morbillivirus and brucella during the 1997-98 health assessment reinforces the need for constant vigilance for these selected agents. As discussed above, the widespread infection with Salmonella among weanling HMS on FFS remains a concern that must be addressed.

This health assessment must be considered as a step in the long-term objective of establishing disease surveillance for the species. In the next phase, temporal trends in many of the parameters can be evaluated. In particular, the differences in morphometrics between FFS and PHR might be used to monitor population trends. It will be important to establish whether some of the perturbations in hematologic and biochemical parameters found represent important signs of population health or merely inherent variability. In this regard, and as an assurance that the pattern of infection with potentially devastating infectious agents is not changing, regular surveillance of the kind conducted under this health assessment activity should be maintained. We recommended regular screening of HMS populations on a biannual basis at this time. In the interim, additional work is needed to clarify the significance of a number of the findings discussed above. This includes attempts at bacterial and viral isolation from animals showing clinical evidence of disease, particularly from aborted fetuses or stillbirths, and full necropsy evaluation of animals if animals are found moribund or recently dead.

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Table 1.--Diagnostic laboratory, serologic tests performed, methods used, antibody titer considered positive, and number of seropositive Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals, Pearl and Hermes Reef and Midway Atoll, 1997-98.

Diagnostic laboratory	Disease agent	Test procedure (positive titer)	No. positive/ No. tested
IDDEX/CVD, Sacramento, CA	*Dirofilaria immitis*	ELISA antigen test	5/105
Oklahoma Animal Disease	*Brucella canis*	Standard Card Agglutination	0/120
Diagnostic Lab, Stillwater, OK	*Brucella abortus*	Standard Card Agglutination	17/120
Federal/State Brucellosis Lab	*Brucella abortus*	Standard Card Agglutination	6/17
	Brucella abortus	PCFIA	12/17
	Brucella abortus	BAPA	5/17
	Brucella abortus	Complement Fixation	2/17
	Brucella abortus	Rivanol	5/17
Oklahoma Animal Disease	Canine distemper virus	Serum neutralization (≥1:8)	0/166
Diagnostic Lab, Stillwater, OK	Dolphin morbillivirus	Serum neutralization (≥1:8)	2/166
	Phocine distemper virus	Serum neutralization (≥1:8)	0/166
	Porpoise morbillivirus	Serum neutralization (≥1:8)	1/166
	Phocine herpes virus 1	Virus neutralization (≥1:8)	0/166
Calicivirus Research Laboratory,	*Leptospira pomona* + 5 serovars	Microscopic agglutination	0/120
OSU, Corvallis, OR	Caliciviruses (32 serotypes)	Serum neutralization	0/120
	California sea lion rotavirus A111R	ELISA (>.200)	0/120
	Marine herpesvirus 206	ELISA (>.200)	2/120
	Human herpesvirus II	ELISA (>.200)	1/120
	Walrus adenovirus W77R	ELISA (>.200)	4/120
	Walrus enterovirus 7-19	ELISA (>.200)	0/120
	Walrus retrovirus T2/19	ELISA (>.200)	1/120
USDA NVSL, Ames, Iowa	*Chlamydia psittaci*	Complement fixation (CF ≥1:20)	46/80
USDA ARS, Beltsville, MD	*Toxoplasma gondii*	Modified agglutination test (≥ 25)	2/118
USDA Foreign Animal Disease	*Brucella abortus*	Standard Card Agglutination	11/80
Diagnostic Lab, Plum Island NY	Canine distemper virus	Serum neutralization (≥1:32)	0/80
	Phocine distemper virus	Serum neutralization (≥1:32)	0/80
	San Miguel Seal Lion Viruses (12)	Serum neutralization	0/80
	Vesicular Exanthema of Swine (12)	Serum neutralization	0/80
	Seal influenza virus	Reference antisera H & N	0/80

Table 2.--Age and gender distribution of Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals (FFS), Pearl and Hermes Reef (PHR) and Midway Atoll (MID), 1997-98.

	FFS		PHR		MID	
	Female	Male	Female	Male	Female	Male
Weanling	4	8	0	0	0	0
Juvenile	5	5	5	10	2	2
Adult	12	22	14	15	5	1
Total	21	35	19	25	7	3

"Juvenile" = juveniles and young subadults (J1, J2, S3)
"Adult" = older subadults and adults (S4, A)

Table 3.--Descriptive statistics for the continuous morphological variables, including dorsal standard length (DSL), axillary girth and weight[1], by gender and size classes of Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals (FFS), Pearl and Hermes Reef (PHR) and Midway Atoll (MID), 1997-98.

Gender	Size	Variable	N	Mean	Std	Median	Minimum	Maximum
FEMALE	Weanling	DSL	4	129.13	7.98	128.75	120.0	139.0
		Girth	4	110.13	8.43	114.00	97.5	115.0
		Weight	4	79.00	15.25	83.50	57.0	92.0
	Juvenile	DSL	12	155.90	12.73	152.13	142.0	185.0
		Girth	12	95.46	7.51	93.50	85.0	108.0
		Weight	12	72.33	16.17	67.00	55.0	106.0
	Adult	DSL	31	211.89	9.83	212.50	183.5	233.0
		Girth	30	137.20	8.81	138.25	119.0	160.0
		Weight	30	200.93	32.31	205.00	140.0	298.0
MALE	Weanling	DSL	8	124.23	4.43	124.15	118.0	130.5
		Girth	8	98.44	6.28	95.75	92.0	108.0
		Weight	8	60.50	9.68	56.50	52.0	76.0
	Juvenile	DSL	17	155.79	10.51	155.00	137.0	178.5
		Girth	17	102.62	7.13	103.50	91.0	117.5
		Weight	17	82.88	16.13	83.00	57.0	123.0
	Adult	DSL	37	203.85	9.43	206.50	171.5	215.0
		Girth	36	131.65	8.83	132.00	111.0	152.0
		Weight	32	177.59	29.00	175.00	106.0	245.0

[1]Weight was calculated based on the formula Axillary girth^2 X Length X 0.00005 (Pitcher, 1986; Arnould, 1995).

Table 4.--Mean differences between *adult female, adult male* and *adult* Hawaiian monk seals (*Monachus schauinslandi*) on French Frigate Shoals (FFS) and Pearl and Hermes Reef (PHR)[1] for selected morphometric continuous variables, 1997-98. *P*-values of the two-sample *t*-test testing for the significance of the differences; and 95% confidence intervals for the difference between the two means.

Variables	PHR mean	FFS mean	Mean diff.[2]	*t*-test *P*-value	95% Confidence Interval	
ADULT FEMALE						
DSL	212.43	207.85	4.57	0.223	-2.98	12.13
Girth	137.07	134.45	2.62	0.444	-4.33	9.57
Weight	201.00	188.45	12.55	0.286	-11.22	36.31
ADULT MALE						
DSL	202.80	204.45	-1.65	0.616	-8.28	4.98
Girth	133.17	130.00	3.17	0.340	-3.55	9.89
Weight	182.00	171.50	10.50	0.332	-11.37	32.37
ADULT						
DSL	207.45	205.69	1.76	0.492	-3.33	6.85
Girth	135.05	131.58	3.47	0.128	-1.02	7.97
Weight	191.17	178.41	12.77	0.109	-2.92	28.45

Table 5. Descriptive statistics for the continuous hematologic values by gender and size classes of Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals (FFS), Pearl and Hermes Reef (PHR), and Midway Atoll (MID), 1997-98.

Gender	Size	Variable	N	Mean	STD	Median	Minimum	Maximum
Female	Weanling	WBC	4	6,366.25	1,629.94	6,270.00	4,840.00	8,085.00
		Neut%	1	65.00	.	65.00	65.00	65.00
		Bands%	1	0.00	.	0.00	0.00	0.00
		Lymph%	1	28.00	.	28.00	28.00	28.00
		Mono%	1	5.00	.	5.00	5.00	5.00
		EOS%	1	2.00	.	2.00	2.00	2.00
		Baso%	1	0.00	.	0.00	0.00	0.00
		PCV	4	50.25	3.30	50.50	46.00	54.00
	Juvenile	WBC	12	7,030.83	1,301.93	7,425.00	4,785.00	9,295.00
		Neut%	12	53.67	8.75	54.00	42.00	70.00
		Bands%	12	0.33	0.78	0.00	0.00	2.00
		Lymph%	12	26.67	5.43	26.00	19.00	38.00
		Mono%	12	5.50	2.32	6.00	2.00	10.00
		EOS%	12	13.50	6.04	14.00	1.00	24.00
		Baso%	12	0.33	0.49	0.00	0.00	1.00
		PCV	12	49.83	4.84	49.00	43.00	59.00
	Adult	WBC	26	8,120.96	2,159.36	8,030.00	4,840.00	12,925.00
		Neut%	30	50.47	8.97	50.50	28.00	67.00
		Bands%	30	0.30	0.60	0.00	0.00	2.00
		Lymph%	30	27.93	6.99	28.00	12.00	42.00
		Mono%	30	6.13	2.65	5.50	1.00	12.00
		EOS%	30	14.63	4.96	14.00	7.00	27.00
		Baso%	30	0.57	0.63	0.50	0.00	2.00
		PCV	31	47.60	6.07	50.00	30.00	55.00
Male	Weanling	WBC	8	6,875.00	1,200.68	6,792.50	5,060.00	8,470.00
		Neut%	7	54.00	9.43	56.00	36.00	62.00
		Bands%	7	0.00	0.00	0.00	0.00	0.00
		Lymph%	7	36.71	8.14	33.00	28.00	52.00
		Mono%	7	5.57	1.13	6.00	4.00	7.00
		EOS%	7	3.57	1.27	4.00	2.00	5.00
		Baso%	7	0.14	0.38	0.00	0.00	1.00
		PCV	8	50.88	3.94	51.50	44.00	57.00
	Juvenile	WBC	17	8,275.88	2,435.82	7,810.00	5,060.00	14,685.00
		Neut%	17	50.29	9.58	50.00	27.00	64.00
		Bands%	17	1.41	1.84	1.00	0.00	6.00
		Lymph%	17	29.06	9.07	29.00	13.00	51.00
		Mono%	17	5.82	2.72	7.00	0.00	10.00
		EOS%	17	12.94	5.54	13.00	6.00	26.00
		Baso%	17	0.47	0.72	0.00	0.00	2.00
		PCV	17	49.94	4.46	51.00	42.00	60.00
	Adult	WBC	32	7,787.66	2,030.41	7,672.50	3,465.00	15,235.00
		Neut%	31	54.48	10.94	53.00	36.00	73.00
		Bands%	31	0.68	0.94	0.00	0.00	3.00
		Lymph%	31	25.77	7.54	26.00	11.00	39.00
		Mono%	31	5.71	2.40	6.00	2.00	10.00
		EOS%	31	12.97	5.94	12.00	0.00	23.00
		Baso%	31	0.39	0.67	0.00	0.00	2.00
		PCV	38	52.18	3.80	52.00	43.00	62.00

Table 6.--Descriptive statistics for the continuous biochemistry values by gender and size classes of Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals (FFS), Pearl and Hermes Reef (PHR) and Midway Atoll (MID), 1997-98.

Gender	Size	Variable	N	Mean	STD	Median	Minimum	Maximum
Female	Weanling	alk. phosphatase	4	367.25	121.04	343.50	255.0	527.0
		SGPT	4	51.75	85.55	11.00	5.0	180.0
		SGOT	4	75.75	89.04	33.50	27.0	209.0
		CPK	4	473.00	243.70	419.50	252.0	801.0
		GGT	4	5.50	3.32	4.50	3.0	10.0
		LDH	4	709.50	214.41	648.00	543.0	999.0
		albumin	4	3.13	0.30	3.20	2.7	3.4
		globulin	4	5.00	0.32	4.95	4.7	5.4
		A/G Ratio	4	0.65	0.06	0.65	0.6	0.7
		protein	4	8.13	0.55	8.25	7.4	8.6
		TS	4	8.50	0.76	8.80	7.4	9.0
		total bilirubin	4	0.30	0.14	0.35	0.1	0.4
		direct bilirubin	4	0.15	0.06	0.15	0.1	0.2
		indirect bilirubin	4	0.15	0.10	0.20	0.0	0.2
		BUN	4	15.00	5.77	15.00	10.0	20.0
		creatinine	4	1.45	0.29	1.45	1.1	1.8
		cholesterol	4	395.75	126.82	396.50	266.0	524.0
		glucose	4	95.00	5.48	93.00	91.0	103.0
		calcium	4	10.25	0.44	10.20	9.8	10.8
		phosphorus	4	7.15	0.37	7.20	6.7	7.5
		bicarbonate	4	32.75	4.57	33.00	27.0	38.0
		chloride	4	101.50	3.11	100.50	99.0	106.0
		potassium	4	4.33	0.46	4.30	3.8	4.9
		sodium	4	148.50	3.00	149.00	145.0	151.0
		B/C ratio	4	11.20	6.06	10.50	5.6	18.2
		Na/K ratio	4	34.50	3.11	34.50	31.0	38.0
		anion gap	4	18.50	4.80	18.00	14.0	24.0
	Juvenile	alk. phosphatase	12	295.75	204.66	232.00	90.0	770.0
		SGPT	12	167.92	68.83	153.00	83.0	295.0
		SGOT	12	108.33	18.60	105.50	80.0	144.0
		CPK	12	867.25	355.61	765.50	407.0	1522.0
		GGT	12	6.75	3.44	7.00	2.0	12.0
		LDH	12	987.42	376.90	845.50	657.0	1884.0
		albumin	12	3.10	0.19	3.10	2.8	3.4
		globulin	12	5.23	0.64	5.25	4.1	6.4
		A/G Ratio	12	0.61	0.10	0.60	0.5	0.8
		protein	12	8.33	0.60	8.35	7.3	9.4
		TS	12	7.86	0.77	7.70	6.8	9.0
		total bilirubin	12	0.17	0.08	0.15	0.1	0.3
		direct bilirubin	12	0.08	0.06	0.10	0.0	0.2
		indirect bilirubin	12	0.08	0.07	0.10	0.0	0.2
		BUN	12	40.00	14.95	45.50	14.0	64.0
		creatinine	12	0.94	0.22	0.90	0.6	1.3
		cholesterol	12	170.33	77.91	167.00	90.0	357.0
		glucose	12	87.50	17.42	88.00	64.0	119.0
		calcium	12	9.88	0.47	9.80	9.4	10.9
		phosphorus	12	6.54	1.89	6.30	4.1	10.2
		bicarbonate	12	29.33	5.21	28.50	21.0	37.0
		chloride	12	104.17	3.61	103.50	98.0	111.0
		potassium	12	4.73	0.42	4.75	3.8	5.2
		sodium	12	149.58	2.23	149.50	146.0	154.0
		B/C ratio	12	47.21	24.05	47.40	11.7	80.0
		Na/K ratio	12	32.00	2.95	31.00	29.0	39.0
		anion gap	12	20.83	4.30	22.50	11.0	26.0

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Table 6.--Cont'd.

Gender	Size	Variable	N	Mean	STD	Median	Minimum	Maximum
Female	Adult	alk. phosphatase	31	258.71	122.65	237.00	124.0	640.0
		SGPT	31	97.19	39.13	92.00	25.0	176.0
		SGOT	31	120.19	52.60	104.00	55.0	252.0
		CPK	31	695.77	335.99	535.00	305.0	1475.0
		GGT	31	6.55	2.88	6.00	1.0	16.0
		LDH	30	944.23	480.98	800.00	380.0	2065.0
		albumin	31	3.13	0.18	3.10	2.7	3.4
		globulin	31	5.26	0.53	5.20	4.3	6.2
		A/G Ratio	31	0.59	0.06	0.60	0.5	0.7
		protein	31	8.40	0.59	8.30	7.3	9.5
		TS	31	8.23	0.71	8.10	6.8	10.2
		total bilirubin	31	0.20	0.10	0.20	0.1	0.5
		direct bilirubin	31	0.10	0.06	0.10	0.0	0.3
		indirect bilirubin	31	0.10	0.07	0.10	0.0	0.3
		BUN	31	33.13	15.59	33.00	9.0	65.0
		creatinine	31	1.30	0.32	1.20	0.9	2.1
		cholesterol	31	224.23	49.92	219.00	138.0	348.0
		glucose	31	93.55	16.93	90.00	61.0	125.0
		calcium	31	9.91	0.70	9.90	8.1	11.3
		phosphorus	31	7.05	1.31	6.80	4.4	9.8
		bicarbonate	31	26.13	4.69	26.00	18.0	36.0
		chloride	31	106.81	3.45	107.00	101.0	116.0
		potassium	31	5.11	0.56	5.00	4.0	6.1
		sodium	31	154.87	3.39	155.00	149.0	161.0
		B/C ratio	31	28.36	16.08	30.00	6.0	58.0
		Na/K ratio	31	30.68	3.19	31.00	26.0	37.0
		anion gap	31	27.06	4.71	27.00	17.0	36.0
Male	Weanling	alk. phosphatase	8	363.13	90.18	378.00	211.0	520.0
		SGPT	8	10.75	3.69	10.00	8.0	19.0
		SGOT	8	26.50	7.91	24.50	19.0	44.0
		CPK	8	390.25	157.76	358.00	221.0	707.0
		GGT	8	3.75	1.58	4.00	2.0	7.0
		LDH	8	606.13	104.42	606.50	463.0	785.0
		albumin	8	3.14	0.17	3.15	2.9	3.3
		globulin	8	4.68	0.21	4.70	4.3	4.9
		A/G Ratio	8	0.69	0.06	0.70	0.6	0.8
		protein	8	7.81	0.20	7.80	7.6	8.2
		TS	8	8.38	0.31	8.30	8.0	9.0
		total bilirubin	8	0.41	0.06	0.40	0.3	0.5
		direct bilirubin	8	0.23	0.05	0.20	0.2	0.3
		indirect bilirubin	8	0.19	0.04	0.20	0.1	0.2
		BUN	8	13.25	4.17	12.00	10.0	23.0
		creatinine	8	1.45	0.17	1.50	1.2	1.7
		cholesterol	8	442.13	61.96	443.50	351.0	552.0
		glucose	8	105.63	7.23	104.00	98.0	121.0
		calcium	8	10.33	0.29	10.20	10.0	10.7
		phosphorus	8	7.40	0.57	7.40	6.4	8.1
		bicarbonate	8	28.13	2.42	28.50	24.0	31.0
		chloride	8	101.63	1.41	101.50	100.0	104.0
		potassium	8	4.18	0.30	4.15	3.7	4.7
		sodium	8	145.50	2.27	145.50	141.0	149.0
		B/C ratio	8	9.46	4.13	7.85	6.7	19.2
		Na/K ratio	8	35.25	2.31	35.50	31.0	38.0
		anion gap	8	19.88	2.17	21.00	17.0	22.0

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Table 6.--Cont'd.

Gender	Size	Variable	N	Mean	STD	Median	Minimum	Maximum
Male	Juvenile	alk. phosphatase	17	375.00	165.11	343.00	187.0	725.0
		SGPT	17	122.59	44.12	115.00	59.0	207.0
		SGOT	17	114.65	41.99	104.00	45.0	220.0
		CPK	17	794.24	372.87	633.00	370.0	1569.0
		GGT	17	7.00	3.35	7.00	1.0	13.0
		LDH	17	1045.18	501.45	921.00	486.0	2227.0
		albumin	17	2.99	0.33	3.00	2.4	3.6
		globulin	17	5.14	0.59	5.30	4.3	6.1
		A/G Ratio	17	0.59	0.10	0.60	0.4	0.7
		protein	17	8.14	0.65	8.30	7.2	9.1
		TS	17	8.15	0.82	8.40	6.8	9.5
		total bilirubin	17	0.22	0.19	0.20	0.1	0.9
		direct bilirubin	17	0.06	0.07	0.10	0.0	0.2
		indirect bilirubin	17	0.16	0.20	0.10	0.0	0.9
		BUN	17	37.41	12.06	37.00	19.0	55.0
		creatinine	17	1.05	0.30	1.00	0.7	1.8
		cholesterol	17	241.18	78.51	233.00	134.0	428.0
		glucose	17	84.41	22.69	90.00	54.0	124.0
		calcium	17	9.92	0.80	9.90	8.5	11.5
		phosphorus	17	7.35	1.22	7.10	5.6	9.9
		bicarbonate	17	26.35	6.14	25.00	18.0	36.0
		chloride	17	104.06	3.65	103.00	98.0	110.0
		potassium	17	4.75	0.33	4.80	4.2	5.2
		sodium	17	148.88	4.03	148.00	143.0	156.0
		B/C ratio	17	39.75	18.58	46.30	13.3	64.3
		Na/K ratio	17	31.41	1.80	31.00	29.0	34.0
		anion gap	17	23.18	6.63	23.00	13.0	34.0
	Adult	alk. phosphatase	37	200.84	96.38	173.00	75.0	427.0
		SGPT	37	84.35	43.58	70.00	19.0	248.0
		SGOT	37	97.73	48.15	82.00	36.0	272.0
		CPK	37	579.30	271.25	483.00	204.0	1270.0
		GGT	37	7.65	2.84	8.00	3.0	18.0
		LDH	36	790.25	331.77	702.50	399.0	1801.0
		albumin	37	3.04	0.29	3.00	2.4	3.6
		globulin	37	5.32	0.52	5.20	4.3	6.7
		A/G Ratio	37	0.59	0.08	0.60	0.4	0.7
		protein	37	8.37	0.62	8.30	7.5	10.1
		TS	38	8.06	0.58	8.05	7.0	9.4
		total bilirubin	37	0.24	0.09	0.20	0.1	0.5
		direct bilirubin	37	0.11	0.06	0.10	0.0	0.2
		indirect bilirubin	37	0.13	0.05	0.10	0.1	0.3
		BUN	37	32.92	16.31	30.00	10.0	64.0
		creatinine	37	1.34	0.34	1.30	0.8	2.2
		cholesterol	37	219.97	55.31	211.00	155.0	344.0
		glucose	37	89.03	13.72	89.00	57.0	120.0
		calcium	37	9.47	0.76	9.40	8.4	11.5
		phosphorus	37	6.24	1.02	6.30	3.8	8.9
		bicarbonate	37	23.54	4.49	23.00	16.0	35.0
		chloride	37	107.46	2.95	107.00	101.0	117.0
		potassium	37	4.61	0.54	4.50	3.9	6.5
		sodium	37	153.11	4.24	153.00	146.0	165.0
		B/C ratio	37	28.04	17.81	23.80	5.3	64.0
		Na/K ratio	37	33.54	3.26	34.00	25.0	41.0
		anion gap	37	26.70	5.78	26.00	16.0	42.0

Table 7.--Mean differences between *female* adult Hawaiian monk seals (*Monachus schauinslandi*) on French Frigate Shoals (FFS) and Pearl and Hermes Reef (PHR)[1] for selected hematologic and biochemistry continuous variables, 1997-98. *P*-values of the two-sample *t*-test testing for the significance of the differences; and 95% confidence intervals for the difference between the two means.

VARIABLES	PHR mean	FFS mean	Mean diff.[2]	*t*-test *P*-value	95% Confidence interval	
WBC	69.30	83.68	-14.38	0.049	-28.66	-0.10
Neut%	49.69	51.83	-2.14	0.582	-10.07	5.78
Lymph%	28.31	26.17	2.14	0.465	-3.82	8.10
Mono%	5.69	6.67	-0.97	0.395	-3.30	1.35
EOS%	15.00	14.83	0.17	0.938	-4.23	4.57
PCV	49.07	48.33	0.74	0.722	-3.49	4.97
Alk. phosphatase	228.64	227.67	0.98	0.973	-57.97	59.93
SGPT	104.29	84.17	20.12	0.178	-9.77	50.01
SGOT	115.36	109.67	5.69	0.767	-34.06	45.44
CPK	743.21	551.00	192.21	0.110	-46.95	431.38
GGT	7.86	5.17	2.69	0.018	0.51	4.87
LDH	1113.64	635.73	477.92	0.005	164.87	790.96
albumin	3.08	3.22	-0.14	0.014	-0.25	-0.03
globulin	5.21	5.17	0.04	0.840	-0.37	0.45
protein	8.29	8.38	-0.10	0.650	-0.54	0.34
TS	8.10	8.36	-0.26	0.325	-0.79	0.27
BUN	40.29	27.00	13.29	0.032	1.26	25.31
creatinine	1.16	1.46	-0.29	0.015	-0.53	-0.06
cholesterol	193.93	253.83	-59.90	0.004	-97.10	-22.71
glucose	89.21	105.17	-15.95	0.009	-27.56	-4.34
calcium	9.91	9.81	0.11	0.700	-0.45	0.67
phosphorus	6.99	6.73	0.27	0.566	-0.68	1.22
bicarbonate	24.86	28.17	-3.31	0.083	-7.09	0.47
chloride	108.21	105.67	2.55	0.051	-0.01	5.11
potassium	5.27	4.70	0.57	0.003	0.22	0.92
sodium	154.86	155.25	-0.39	0.778	-3.23	2.45
B/C ratio	36.13	20.98	15.15	0.015	3.16	27.13
Na/K ratio	29.50	33.25	-3.75	0.001	-5.71	-1.79
anion gap	27.14	26.08	1.06	0.559	-2.63	4.75

[1]Only adults (i.e., older subadults and adults) were used due to the small number of weanlings and juveniles. Only the islands FFS and PHR were compared due to the small number of animals from Midway.

[2]Mean for PHR minus mean for FFS.

Table 8.-Mean differences between *male* adult Hawaiian monk seals (*Monachus schauinslandi*) on French Frigate Shoals (FFS) and Pearl and Hermes Reef (PHR)[1] for selected hematologic and biochemistry continuous variables, 1997-98. *P*-values of the two-sample *t*-test testing for the significance of the differences; and 95% confidence intervals for the difference between the two means.

Variables	PHR mean	FFS mean	Mean diff.[2]	*t*-test *P*-value	95% Confidence interval	
WBC	78.96	79.52	-0.56	0.937	-14.84	13.72
Neutrophils %	45.53	63.07	-17.53	0.000	-22.53	-12.53
Lymphocytes %	31.13	20.20	10.93	0.000	6.93	14.93
Monocytes %	5.33	6.27	-0.93	0.292	-2.71	0.84
Eosinophils %	16.67	9.60	7.07	0.000	3.44	10.69
Packed Cell Volume	51.40	52.73	-1.33	0.310	-3.94	1.29
alk. phosphatase	230.47	175.95	54.51	0.096	-10.25	119.28
SGPT	82.13	84.57	-2.44	0.859	-30.13	25.25
SGOT	92.47	97.71	-5.25	0.714	-34.23	23.74
CPK	563.07	590.24	-27.17	0.775	-218.68	164.34
GGT	6.80	8.19	-1.39	0.155	-3.33	0.55
LDH	819.33	770.90	48.43	0.680	-188.30	285.17
albumin	2.93	3.10	-0.17	0.057	-0.34	0.00
globulin	5.33	5.29	0.05	0.789	-0.31	0.41
protein	8.27	8.39	-0.12	0.562	-0.53	0.29
TS	8.12	7.96	0.16	0.385	-0.21	0.53
BUN	47.33	21.90	25.43	0.000	18.28	32.58
creatinine	1.09	1.51	-0.42	0.000	-0.59	-0.24
cholesterol	230.47	208.90	21.56	0.248	-15.75	58.87
glucose	88.33	89.05	-0.71	0.881	-10.34	8.91
calcium	9.69	9.23	0.45	0.038	0.03	0.88
phosphorus	6.21	6.16	0.04	0.894	-0.63	0.72
bicarbonate	23.07	23.95	-0.89	0.572	-4.04	2.27
chloride	108.40	106.71	1.69	0.095	-0.31	3.68
potassium	4.85	4.35	0.51	0.000	0.25	0.76
sodium	153.53	152.24	1.30	0.319	-1.31	3.90
B/C ratio	44.43	16.23	28.19	0.000	20.33	36.06
Na/K ratio	31.73	35.24	-3.50	0.000	-5.17	-1.84
anion gap	27.00	25.81	1.19	0.476	-2.16	4.54

[1]Only adults (i.e., older subadults and adults) were used due to the small number of weanlings and juveniles. Only the islands FFS and PHR were compared due to the small number of animals from Midway.
[2]Mean for PHR minus mean for FFS .

Table 9.--Mean differences between adult Hawaiian monk seals (*Monachus schauinslandi*) on French Frigate Shoals (FFS) and Pearl and Hermes Reef (PHR) for selected hematologic and biochemistry continuous variables, 1997-98. *P*-values of the two-sample *t*-test testing for the significance of the differences; and 95% confidence intervals for the difference between the two means.

Variables	PHR mean	FFS mean	Mean diff.[1]	*t*-test *P*-value	95% Confidence interval	
Monocytes %	5.50	6.44	-0.94	0.174	-2.32	0.43
Packed cell volume	50.28	51.18	-0.90	0.453	-3.29	1.49
Albumin	3.00	3.14	-0.14	0.018	-0.25	-0.02
Protein	8.28	8.38	-0.11	0.453	-0.40	0.18
BUN	43.93	23.76	20.17	0.000	13.77	26.58
Creatinine	1.13	1.49	-0.36	0.000	-0.50	-0.23
Calcium	9.80	9.44	0.35	0.049	0.00	0.71
Chloride	108.31	106.33	1.98	0.012	0.46	3.50
Potassium	5.06	4.48	0.58	0.000	0.36	0.80
B/C ratio	40.42	17.96	22.46	0.000	15.77	29.15
Na/K ratio	30.66	34.52	-3.86	0.000	-5.19	-2.53
Anion gap	27.07	25.91	1.16	0.349	-1.30	3.62

[1]Mean for PHR minus mean for FFS.

Table 10.--Suspect and positive serologic testing results against *Brucella abortus* from 120 serum specimens collected from Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals (FFS), Pearl and Hermes Reef (PHR) and Midway Atoll (MID), 1997-98.

Date	Seal ID	Sex	Age	Site	FADDL card	OADDL card	NVLS card	PCFIA	BAPA	CF	Rivanol
970321	Y654	F	A	FFS	Neg	Pos	NT	0.577/Pos	Neg 200	AC	NT
980709	Y376	M	A	FFS	Pos	Pos	Pos	0.133/Neg	NT	NT	100
980711	YY26	M	W	FFS	Neg	Pos	Pos	0.182/Neg	NT	NT	100
980713	YY39	M	W	FFS	Pos	Neg	NT	NT	NT	NT	NT
980719	Y746	M	A	FFS	Pos	Pos	Pos	0.179/Neg	NT	NT	25
971027	BL34	F	A	PHR	Neg	Pos	NT	0.381/Pos	Neg 200	AC	NT
971028	BC05	M	J1	PHR	Neg	Pos	NT	0.299/Pos	Pos 100	AC	NT
971028	BN48	M	A	PHR	Pos	Pos	NT	0.368/Pos	Pos 200	AC	NT
971101	B6AA	F	A	PHR	Neg	Pos	NT	0.374/Pos	Pos 100	AC	NT
971101	R2AG	F	A	PHR	Pos	Pos	NT	0.308/Pos	Pos 100	AC	NT
980216	R2AG	F	A	PHR	Pos	Pos	NT	0.218/Pos	Neg 200	AC	NT
980217	BC05	M	J2	PHR	Pos	Pos	NT	0.340/Pos	Pos 100	AC	NT
980219	BK11	F	A	PHR	Neg	Pos	NT	0.436/Pos	Pos 100	AC	NT
980222	YS29	F	A	PHR	Pos	Pos	NT	0.317/Pos	Pos 25	AC	NT
980626	YZ03	F	A	MID	Neg	Pos	Atypical	0.371/Pos	Pos 50	164 AC4	25
980627	BK27	F	A	MID	Pos	Pos	Atypical	0.197/Neg	Pos 50	164 AC4	100
980628	BX22	F	S3	MID	Pos	Pos	Atypical	0.688/Pos	Pos 50	83 AC4	Neg
980626	RZ02	F	A	MID	Pos	Neg	NT	NT	NT	NT	NT

FADDL--Foreign Animal Disease diagnostic Laboratory, Plum Island, New York
OADDL--Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma
NVLS--National Veterinary Laboratory Services, Ames, Iowa
Card--Standard card agglutination test
PCFIA--Particle concentration fluorescence immunoassay
BAPA--Buffered antigen standard plate agglutination test
CF--Complement fixation test
FFS--French Frigate Shoals
PHR--Pearl and Hermes Reef
MID--Midway Atoll
Neg--Negative
Pos--Positive
NT--Not tested
AC--Anticomplementary results

Table 11.--*Salmonella* spp. isolates identified from 62 specimens collected Hawaiian monk seals (*Monachus schauinslandi*) sampled at French Frigate Shoals (FFS), Pearl and Hermes Reef (PHR) and Midway Atoll (MID), 1997-98.

Date	Seal ID	Sex	Size	Location	Isolate
980217	BA70	M	A	PHR	*Salmonella* spp.
980711	YY26	M	W	FFS	*Salmonella chester*
980711	YY38	M	W	FFS	*Salmonella san diego*
980712	YY11	M	W	FFS	*Salmonella* spp.
980712	YY12	M	W	FFS	*Salmonella minnesota*
980712	YY19	M	W	FFS	*Salmonella oranienburg*
980712	YY28	F	W	FFS	*Salmonella oranienburg*
980712	YY30	M	W	FFS	*Salmonella minnesota*
980713	YY13	M	W	FFS	*Salmonella minnesota*
980713	YY22	F	W	FFS	*Salmonella oranienburg. Salmonella minnesota*
980713	YY39	M	W	FFS	*Salmonella cerro Salmonella minnesota*
980714	YY33	F	W	FFS	*Salmonella chester*
980714	YY40	F	W	FFS	*Salmonella chester*
980816	RY24	M	W	MID	*Salmonella* group E

Table 12.--Prevalence and intensity of helminth ova/smear in PVA fixed fecal loop specimens collected from Pearl and Hermes Reef, Midway Atoll and French Frigate Shoals, 1998. Hawaiian monk seal populations about 150, 50 and 350, respectively.

Parasite species	PHR			MID			FFS		
	n+/n	mean	range	n+/n	mean	range	n+/n	mean	range
Contracaecum Ova	8/19	5.6	0-24	3/10	2.6	0-12	5/10	9.1	0-30
Diphyllobothrium Ova	19/19	2011	65-10980	7/10	912	0-4760	8/10	871	0-4530
Corynosoma (?)	2/19	0.7		1/10	0.1	0-1	0/10	0.0	
Acanthocephala Ova	2/19	0.7	0-12*	1/10	0.1	0-1	2/10	3.3	0-30
Protozoa	0/16	0.0		0/10	0.0		0/10	0.0	